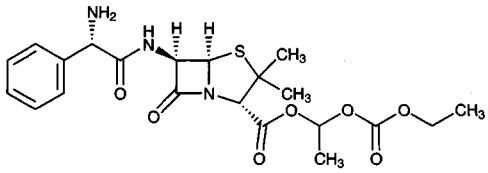
Bacampicillin



Molecular formula: $C_{21}H_{27}N_3O_7S$
Molecular weight: 465.53
CAS Registry No.: 50972-17-3, 37661-08-8 (HCl)
Merck Index: 961
Lednicer No.: 3 204

SAMPLE

Matrix: blood
Sample preparation: Add 100000 fold excess of ampicillin to eliminate adsorption of bacampicillin, extract into butyl acetate, re-extract into pH 2 buffer. Wash the aqueous phase with n-hexane, inject an aliquot.

HPLC VARIABLES

Column: 100 × 2.9 5 μm Nucleosil C18
Mobile phase: MeCN:pH 7.4 phosphate buffer 41:49
Injection volume: 20
Detector: F with post-column reaction. The column effluent mixed with two volumes of sodium borate buffer then with one volume of 150 μg/mL fluorescamine in acetone (Science 1972, 178, 871).

CHROMATOGRAM

Limit of detection: 800 pg/mL

REFERENCE

Sjövall,J.; Westerlund,D.; Alván,G.; Magni,L.; Nord,C.E.; Sörstad,J. Rectal bioavailability of bacampicillin hydrochloride in man as determined by reversed-phase liquid chromatography, *Chemotherapy*, **1984**, *30*, 137–147.

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 1 mL MeOH, stir for 5 min, centrifuge at 2400 g for 10 min. Remove 1 mL supernatant, add 2 μg cefazolin, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 20:80
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM

Retention time: 9 (measured as ampicillin peak)
Internal standard: cefazolin (14)
Limit of detection: 500 ng/mL

KEY WORDS

plasma

REFERENCE

Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E.; Picari,M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin, *J.Chromatogr.*, **1990**, *507*, 235–239.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.687

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

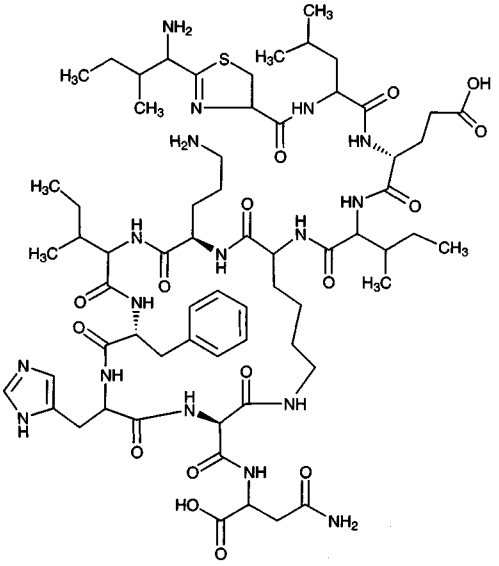
Bacitracin

Molecular formula: $C_{66}H_{103}N_{17}O_{16}S$ (bacitracin A)

Molecular weight: 1422.61 (bacitracin A)

CAS Registry No.: 1405-87-4, 1405-89-6 (zinc salt), 1405-88-5 (methylenedisalicylate)

Merck Index: 965



SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 g in 100 mL 20 mM HCl in MeOH:water 80:20, mix, vortex, centrifuge, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC basic C8 200 Å (YMC)
Mobile phase: Gradient. MeOH:50 mM pH 6.5 KH_2PO_4 from 57:43 to 63:37 over 1 h
Column temperature: 25
Flow rate: 1
Injection volume: 100
Detector: UV 215

CHROMATOGRAM

Retention time: 14, 16, 20, 21, 25, 27, 30 (bioactive fractions)

REFERENCE

Bell,R.G. Preparative high-performance liquid chromatographic separation and isolation of bacitracin components and their relationship to microbiological activity, *J.Chromatogr.*, **1992**, *590*, 163–168.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in saline at a concentration of 1-10 mg/mL, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 C18 (Vydac)
Mobile phase: Gradient. MeCN:water (both containing 0.05% trifluoroacetic acid) from 5:95 to 65:35 over 20 min
Flow rate: 2
Detector: UV 210

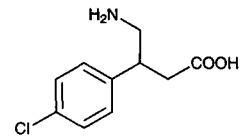
KEY WORDS

saline

REFERENCE

Drapeau,G.; Petitclerc,E.; Toulouse,A.; Marceau,F. Dissociation of the antimicrobial activity of bacitracin USP from its renovascular effects, *Antimicrob.Agents Chemother.*, **1992**, *36*, 955–961.

Baclofen



Molecular formula: $C_{10}H_{12}ClNO_2$
Molecular weight: 213.66
CAS Registry No.: 1134-47-0
Merck Index: 967
Lednicer No.: 2 121

SAMPLE

Matrix: CSF

Sample preparation: Prepare a 100 × 7 column of Dowex 50X4-400, treat with an excess of aqueous ammonia, wash with water until the washings are neutral, wash with an excess of 4 M HCl, wash with water until the washings are neutral, rinse with 8 mL water. Add 200 μL CSF to the column, wash with 8 mL water, elute with 2 mL 10% ammonia. Lyophilize the eluate, reconstitute the residue with 1 mL pH 7.4 phosphate buffer, extract twice with 1 mL portions of 1-butanol. Combine the organic layers and evaporate them to dryness, add 10-50 μL EtOH:water:triethylamine 50:25:25, evaporate to dryness, add 20 μL reagent, let stand at room temperature for 20 min, evaporate to dryness, reconstitute with pH 7.4 sodium phosphate buffer, inject a 5-25 μL aliquot. (Reagent was EtOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, store at -20° (Anal.Biochem. 1989, 176, 269).)

HPLC VARIABLES

Column: 150 × 3.9 Pico-Tag
Mobile phase: Gradient. A was sodium acetate adjusted to pH 6.4 with glacial acetic acid. B was MeCN:water 60:40. A:B from 100:0 to 60:40 over 10 min, to 0:100 over 1 min, maintain at 0:100 over 3 min.
Column temperature: 38
Injection volume: 5-25
Detector: UV 254

CHROMATOGRAM

Retention time: 13.2
Limit of detection: 5-10 ng/mL

KEY WORDS

derivatization; SPE; pharmacokinetics

REFERENCE

Sallerin-Caute,B.; Monsarrat,B.; Lazorthes,Y.; Cros,J.; Bastide,R. A sensitive method for the determination of baclofen in human CSF by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1988**, *11*, 1753–1761.

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut SCX strong cation exchange SPE cartridge with 2 mL hexane, 2 mL MeOH, 2 mL water, and 3 mL saturated NaCl solution. 1 mL Plasma + 100 μL water + 1 mL citrate buffer, mix, add to the SPE cartridge, wash with 4 mL water, wash with 1 mL saturated NaCl solution, dry the SPE cartridge, elute with 1.5 mL pH 10.4 borate buffer. Mix a 200 μL aliquot of the eluate and add it to 50 μL reagent, mix, inject a 20 μL aliquot. (Prepare citrate buffer by diluting 89 mL 100 mM citric acid to 100 mL with 200 mM Na_2HPO_4, pH 2.6. Prepare pH 10.4 borate buffer by mixing 54 mL 200 mM boric acid in 100 mM NaOH with 46 mL 100 mM NaOH. Prepare pH 9.3 borate buffer by mixing 87 mL 200 mM boric acid in 100 mM NaOH with

13 mL 100 mM NaOH. Prepare reagent daily by mixing 75 mg o-phthalaldehyde, 5 mL MeOH, 50 μL tert-butanethiol, and 5 mL pH 9.3 borate buffer.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak
Mobile phase: MeOH:buffer 74:36 (Prepare buffer by adjusting the pH of 60 mM Na_2HPO_4 to 7 with 60 mM KH_2PO_4.)
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5020 guard cell +1.2 V, Model 5011 glassy carbon working cell, screen electrode +0.2 V, quantifying electrode +0.7 V

CHROMATOGRAM
Retention time: 26
Limit of detection: 2.5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
derivatization; plasma; SPE; pharmacokinetics

REFERENCE
Millerioux,L.; Brault,M.; Gualano,V.; Mignot,A. High-performance liquid chromatographic determination of baclofen in human plasma, *J.Chromatogr.A*, **1996**, *729*, 309–314.

SAMPLE
Matrix: blood, CSF, dialysate, tissue
Sample preparation: Dilute dialysate and CSF. Homogenize brain tissue with 4 volumes of MeOH:water in an ice bath, let stand at -20° for 8 h. 30 μL Plasma + 60 μL MeOH, mix, let stand at -20° for 1 h, centrifuge at 4° at 10000 rpm for 5 min, dilute the supernatant with water. Mix an aliquot of tissue homogenate, deproteinized plasma, diluted dialysate, or diluted CSF with an equal volume of the reagent, let stand for 1.5 min, inject a 30 μL aliquot. (Prepare reagent by mixing 50 mg o-phthalaldehyde, 900 μL MeOH, 100 μL 400 mM pH 9.2 borate buffer, and 50 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 250 × 4.6 5 μm Finepak SIL C18S ODS (Jasco)
Mobile phase: MeOH:THF:100 mM pH 6.95 acetate buffer 45.5:2:52.5 (dialysate, CSF, plasma) or 43:2:55 (tissue)
Flow rate: 1
Injection volume: 30
Detector: F ex 368 em 434

CHROMATOGRAM
Limit of detection: 100 nM

KEY WORDS
plasma; derivatization; rat; brain; pharmacokinetics

REFERENCE
Deguchi,Y.; Inabe,K.; Tomiyasu,K.; Nozawa,K.; Yamada,S.; Kimura,R. Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis, *Pharm.Res.*, **1995**, *12*, 1838–1844.

SAMPLE
Matrix: blood, urine

Sample preparation: 500 μL Plasma + 2 mL MeOH, centrifuge. 1 mL Supernatant or 100 μL urine + 500 μL pH 9 sodium tetraborate buffer + 250 μL 0.2% 4-chloro-7-nitrobenzofurazan in MeOH, heat at 60° for 45 min, acidify with 100 mM HCl, extract with 5 mL ethyl acetate. Evaporate 3 mL of the extract to about 500 μL, dry with anhydrous sodium sulfate, pass through SPE column, elute with ethyl acetate to give a final volume of 2 mL. Evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 10 μL aliquot. (Prepare the 25 × 6 SPE column with 0.063-0.200 mm silica gel (Merck), wet it with ethyl acetate.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeOH:water 45:55
Flow rate: 0.4
Injection volume: 10
Detector: F ex 463 em 524

CHROMATOGRAM
Retention time: 4.0
Limit of detection: 100 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics; SPE; derivatization

REFERENCE
Tosunoglu,S.; Ersoy,L. Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection, *Analyst*, **1995**, *120*, 373–375.

SAMPLE
Matrix: formulations
Sample preparation: Dilute an aliquot with water to a concentration of 5 μg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Speri-5 ODS (Applied Biosystems)
Mobile phase: MeCN:50 mM NaH_2PO_4 20:80, adjusted to pH 3.5 with 85% phosphoric acid
Flow rate: 1
Injection volume: 15
Detector: UV 220

CHROMATOGRAM
Retention time: 5.5

KEY WORDS
syrup; stability-indicating

REFERENCE
Johnson,C.E.; Hart,S.M. Stability of an extemporaneously compounded baclofen oral liquid, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2353–2355.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:10 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:MeCN:2.72 g/L KH_2PO_4 2:12:86

Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 10.3

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 5 μm Adsorbosphere C18
Mobile phase: MeOH:buffer 40:60 (Buffer was 50 mM sodium acetate adjusted to pH 6.4 with glacial acetic acid.)
Flow rate: 1.0
Injection volume: 100
Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 10 μg/mL

REFERENCE
A simple, rapid and reliable HPLC method for the analysis of baclofen in tablets (Abstract 3350), *Pharm.Res.*, **1997**, *14*, S581–S581.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer: 25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: amphetamine, tranylcypromine

KEY WORDS
derivatization

REFERENCE

Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE

Matrix: solutions

Sample preparation: 200 µL 10 mM Baclofen in 100 mM sodium bicarbonate + 200 µL 10 mM 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (freshly prepared), stir at 40° for 1 h, cool, add 100 µL 200 mM HCl, inject an aliquot. (Derivatization in Anal.Biochem. 1992, 202, 210.)

HPLC VARIABLES

Column: 150 × 4.6 5 µm YMC.GEL C8 (YMC)
Mobile phase: MeOH:5% KH_2PO_4 (pH 4.0) 11:8
Flow rate: 1
Detector: UV 340

CHROMATOGRAM

Retention time: 17 (R-(-)), 20 (S-(+))

KEY WORDS

chiral; derivatization

REFERENCE

Shimada,K.; Mitamura,K.; Morita,M.; Hirakata,K. Separation of the diastereomers of baclofen by high performance liquid chromatography using cyclodextrin as a mobile phase additive, *J.Liq. Chromatogr.*, **1993**, *16*, 3311–3320.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.71 (A), 3.47 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, fu-

rosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

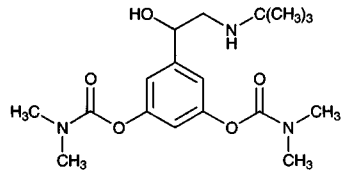
CHROMATOGRAM
Retention time: 9.32, 14.23

OTHER SUBSTANCES
Simultaneous: levodopa, norephedrine, primaquine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Bambuterol



Molecular formula: $C_{18}H_{29}N_3O_5$
Molecular weight: 367.45
CAS Registry No.: 81732-65-2, 81732-46-9 (HCl)
Merck Index: 980

SAMPLE

Matrix: blood
Sample preparation: Dilute plasma with buffer containing IS, inject an aliquot corresponding to 0.5 μL plasma.

HPLC VARIABLES

Guard column: 4 × 4 LiChrosorb RP-select B
Column: 50 × 4 5 μm LiChrospher RP-select B
Mobile phase: Gradient. MeOH:100 mM pH 5 ammonium acetate from 3:97 to 10:90 over 5 min, to 25:75 over 1 min, to 38:62 over 7 min
Flow rate: 1.4
Detector: MS, thermospray, Finnigan 4500 quadrupole, ion source repeller equipped with polyimide sleeve, trap at -90°, analyzer pressure 0.000035 Torr, manifold fore pressure 0.20 Torr, exhaust line pressure 1.1 Torr, repeller 45 V, vaporizer 115°, jet 200°, aerosol 270

CHROMATOGRAM

Retention time: 11
Internal standard: hedxadeuterobambuterol

KEY WORDS

plasma; LC-MS; dog

REFERENCE

Lindberg,C.; Paulson,J.; Blomqvist,A. Evaluation of an automated thermospray liquid chromatography-mass spectrometry system for quantitative use in bioanalytical chemistry, *J.Chromatogr.*, **1991**, *554*, 215–226.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with three 3 mL portions of EtOH, two 3 mL portions of water, and with 3 mL 10 mM pH 7.5 phosphate buffer. Add the incubation mixture to the SPE cartridge, wash with two 3 mL portions of water, elute with two 1 mL portions of EtOH:50 mM pH 8.5 ammonium chloride buffer 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 500 μL of the initial mobile phase, inject a 200 μL aliquot.; SPE

HPLC VARIABLES

Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 250 mM pH 4.6 ammonium acetate buffer. B was MeCN: 500 mM pH 4.6 ammonium acetate buffer 50:50. From A:B 90:10 to 10:90 over 20 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM

Retention time: 16.5

OTHER SUBSTANCES
Extracted: terbutaline, metabolites

KEY WORDS
rat; SPE

REFERENCE
Lindberg,C.; Roos,C.; Tunek,A.; Svensson,L.Å. Metabolism of bambuterol in rat liver microsomes: identification of hydroxylated and demethylated products by liquid chromatography mass spectrometry, *Drug Metab.Dispos.*, **1989**, *17*, 311–322.

SAMPLE
Matrix: perfusate, tissue
Sample preparation: Perfusate. 400 μL Lung perfusate + 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot of the supernatant. Tissue. Homogenize lung in 2 volumes of water (Polytron Homogenizer), mix 400 μL homogenate with 400 μL 5% perchloric acid, mix, centrifuge, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 5 Nucleosil 10SA
Mobile phase: Gradient. A was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L water. B was 19.3 g ammonium acetate and 14.4 mL acetic acid in 1 L MeCN:water 50:50. A:B from 90:10 to 10:90 over 20 min, stay at 10:90 for 3 min, return to initial conditions over 3 min, re-equilibrate for 7 min.
Flow rate: 1
Injection volume: 200
Detector: UV 254

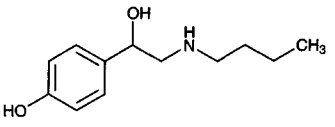
CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: metabolites, terbutaline

KEY WORDS
lung; guinea pig

REFERENCE
Ryrfeldt,Å.; Nilsson,E.; Tunek,A.; Svensson,L.-Å. Bambuterol: uptake and metabolism in guinea pig isolated lungs, *Pharm.Res.*, **1988**, *5*, 151–155.

Bamethan



Molecular formula: $C_{12}H_{19}NO_2$
Molecular weight: 209.29
CAS Registry No.: 3703-79-5, 5716-20-1 (sulfate)
Merck Index: 981
Lednicer No.: 2 39

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 5.91

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

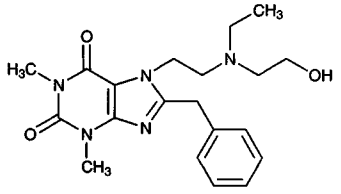
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Bamifylline



Molecular formula: $C_{20}H_{27}N_5O_3$
Molecular weight: 385.47
CAS Registry No.: 2016-63-9, 20684-06-4 (HCl)
Merck Index: 982
Lednicer No.: 1 426

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 10.288

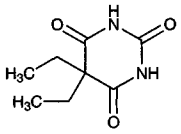
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Barbital



Molecular formula: $C_8H_{12}N_2O_3$
Molecular weight: 184.19
CAS Registry No.: 57-44-3, 144-02-5 (sodium salt)
Merck Index: 989
Lednicer No.: 1 267

SAMPLE

Matrix: blood
Sample preparation: Inject a 5-20 μL aliquot of serum directly onto the column with mobile phase A or B.

HPLC VARIABLES

Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 8:92 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B)
Flow rate: 1
Injection volume: 5 (A), 20 (B)
Detector: UV 254

CHROMATOGRAM

Retention time: 7.60 (A), 8 (B)

OTHER SUBSTANCES

Simultaneous: acetaminophen (B), carbamazepine (B), phenobarbital (B), phenytoin (B), primidone (B), sulfapyridine (B)

KEY WORDS

serum

REFERENCE

Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

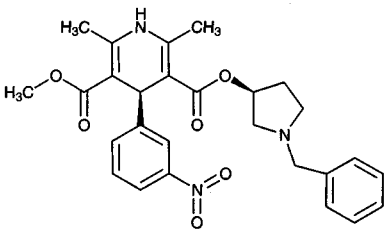
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Barnidipine



Molecular formula: $C_{27}H_{29}N_3O_6$
Molecular weight: 491.54
CAS Registry No.: 104713-75-9
Merck Index: 1031

SAMPLE

Matrix: bile, urine
Sample preparation: Inject urine and bile directly. Hydrolyse urine or bile by heating a 1 mL aliquot with 200 µL 200 mM pH 5.5 sodium acetate buffer and 20 µL 100 U/mL β-glucuronidase at 37° for 24 h, add to a Sep-Pak C18 SPE cartridge, elute with MeOH. Evaporate the eluate, reconstitute the residue with MeOH:50 mM pH 5.0 sodium acetate buffer 5:95, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: Gradient. MeOH:50 mM pH 5.0 sodium acetate buffer 5:95 for 5 min, to 50:50 over 95 min
Flow rate: 0.8
Detector: UV

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; SPE

REFERENCE

Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

SAMPLE

Matrix: blood
Sample preparation: 500 µL Serum or plasma + 500 µL 500 mM pH 11 phosphate buffer + 3 mL benzene (Caution! Benzene is a carcinogen!), shake mechanically for 5 min, centrifuge at 3000 rpm for 10 min. Remove 2.7 mL of the organic layer and add it to 50 ng IS, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 75 µL mobile phase, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Aluspher RP-select B (Merck)
Mobile phase: MeCN:100 mM pH 11.8 Britton-Robinson buffer 40:60
Flow rate: 0.9
Injection volume: 5
Detector: E, JASCO Model 840-EC, glassy carbon working electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5
Internal standard: 2-(N-benzyl-N-methylamino) ethylmethyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate hydrochloride (YC-204, Yamanouchi) (7.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: acetazolamide, allopurinol, amitriptyline, chlorpromazine, clonidine, cyproheptadine, hydrochlorothiazide, imipramine, methyldopa, phenoxybenzamine, propranolol, ascorbic acid

KEY WORDS
human; dog; serum; plasma; protect from light; pharmacokinetics

REFERENCE
Takamura,K.; Abdel-Wadood,H.M.; Kusu,F.; Rafaat,I.H.; Saleh,G.A.; El-Rabbat,N.A.; Otagiri,M. Determination of barnidipine in human serum and dog plasma by HPLC with electrochemical detection, *Biol.Pharm.Bull.*, **1995**, *18*, 1311–1314.

SAMPLE
Matrix: blood, enzyme incubations
Sample preparation: 1 mL Enzyme incubation or plasma + 5 mL diethyl ether + 500 μL saturated sodium bicarbonate, extract, centrifuge at 800 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeOH:20 mM pH 3.0 bromo-tetra-n-propylammonium phosphate 50:50
Flow rate: 1
Detector: UV

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog; plasma

REFERENCE
Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL ice-cold MeOH, vortex, centrifuge at 1000 g for 15 min, filter (0.22 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: Gradient. MeCN:20 mM pH 7.0 ammonium acetate buffer 30:70 for 5 min, to 70:30 over 55 min, maintain at 70:30 for 30 min.
Flow rate: 1
Detector: radioactivity

CHROMATOGRAM
Retention time: 66

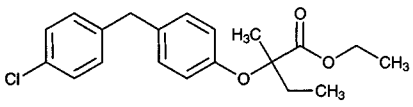
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; dog; liver; ^{14}C labeled

REFERENCE

Teramura,T.; Tokunaga,T.; Matsumoto,H.; Watanabe,T.; Higuchi,S. Metabolism of barnidipine hydrochloride, a potent calcium antagonist, in rat and dog, *Xenobiotica*, **1996**, *26*, 177–187.

Beclobrate



Molecular formula: $C_{20}H_{23}ClO_3$
Molecular weight: 346.85
CAS Registry No.: 55937-99-0
Merck Index: 1046

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL acetic acid + 1.75 mL 10 μg/mL IS in hexane, shake for 5 min, centrifuge at 2000 rpm for 20 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 30 × 4 30-40 μm RP18 (E. Merck)
Column: 125 × 4 5 μm LiChrosorb RP18
Mobile phase: MeCN:MeOH:water:acetic acid 40:30:30:0.1
Flow rate: 2
Injection volume: 50
Detector: UV 27

CHROMATOGRAM

Retention time: 4.1 (beclobric acid)
Internal standard: SGD 2774 (5.8)

KEY WORDS

plasma; pharmacokinetics; bioequivalence

REFERENCE

Gikalov,I.; Ifflaender,U. Pharmacokinetik und Bioäquivalenz von zwei peroralen Beclobrat-Zubereitungen [Pharmacokinetics and bioequivalence of two peroral beclobrate preparations], *Arzneimittelforschung*, **1987**, *37*, 1065–1068.

SAMPLE

Matrix: blood, urine
Sample preparation: 200 μL Plasma or 100 μL urine + 500 (plasma) or 300 (urine) mg NaCl + 50 μL 1 μg/mL clofibric acid in MeOH + 1 (plasma) or 0.3 (urine) mL pH 4 buffer + 5 mL n-hexane:EtOH 90:10, shake horizontally for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, add 50 μL toluene and evaporate it to remove traces of water. Reconstitute the residue in 500 μL dichloromethane, add 50 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane: pyridine 99:1, add 50 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 50 μL 1 mg/mL FLOPA, vortex, let stand at room temperature for 2 h, evaporate to dryness, reconstitute in 500 μL mobile phase, inject a 50 μL aliquot. (FLOPA is the corresponding amine hydrochloride from (+)-(S)-flunoxaprofen. Synthesis is as follows (protect from light). 500 mg (+)-(S)-Flunoxaprofen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, add 600 mg sodium azide dissolved in ice water with stirring, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate,

evaporate to dryness, dissolve in 1 mL toluene, evaporate to give crystals (mp 91°). Dissolve in ether, add 0.5 M HCl in ether, filter, dissolve solid in a small volume of MeOH, precipitate with ether, dry FLOPA over phosphorus pentoxide under vacuum (Pharm.Res. 1990, 7, 1262).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: Gradient. A was n-hexane:chloroform:EtOH 100:10:0.75. B was n-hexane: chloroform:EtOH 100:10:20. A:B 100:0 for 10 min, 50:50 for 5 min, 100:0 for 5 min (stepwise).
Flow rate: 2
Injection volume: 50
Detector: F ex 305 em 355

CHROMATOGRAM

Retention time: 6 (-), 6.5 (+)
Internal standard: clofibric acid (8)
Limit of detection: 25 ng/mL

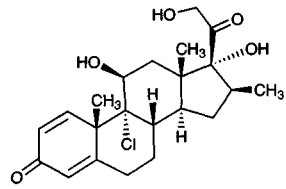
KEY WORDS

plasma; pharmacokinetics; chiral; derivatization; normal phase

REFERENCE

Mayer,S.; Mutschler,E.; Spahn-Langguth,H. Pharmacokinetic studies with the lipid-regulating agent beclobrate: enantiospecific assay for beclobric acid using a new fluorescent chiral coupling component (S-FLOPA), *Chirality*, **1991**, *3*, 35–42.

Beclomethasone



Molecular formula: $C_{22}H_{29}ClO_5$
Molecular weight: 408.92
CAS Registry No.: 4419-39-0, 5534-09-8 (beclomethasone dipropionate)
Merck Index: 1047

SAMPLE

Matrix: blood
Sample preparation: Add 30 mL EtOH to 50 mL plasma, extract with dichloromethane for 15 min, centrifuge at 2500 rpm for 5 min. Evaporate dichloromethane layer to dryness under vacuum at 30°, reconstitute residue in mobile phase and inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima C18 (Alltech Associates, Australia) (A) or 250 × 4.6 5 μm Econosphere C18 (Alltech Associates, Australia) (B) or 150 × 4.6 5 μm Ultrasphere C8 (C)
Mobile phase: MeOH:MeCN:acetic acid:water 30.9:14.6:4.4:0.1 (A) or 25:20.5:4.4:0.1 (B) or 27.5:18:4.4:0.1 (C)
Flow rate: 1.3 (A, C) or 1.2 (B)
Injection volume: 100
Detector: UV 242

CHROMATOGRAM

Retention time: 4.9 (A), 5.5 (B), 3.3 (C) (beclomethasone); 23.0 (A), 25.9 (B), 17.6 (C) (beclomethasone dipropionate)

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

plasma

REFERENCE

Foe,K.; Cheung,H.T.A.; Tattam,B.N.; Brown,K.F.; Seale,J.P. Degradation products of beclomethasone dipropionate in human plasma, *Drug Metab.Dispos.*, **1998**, *26*, 132–137.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 300 μL water + 6 mL diethyl ether, shake for 5 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen. Dissolve in 100 μL MeOH. Add 20 μL copper acetate solution and let stand at room temperature for 1 h. Add 80 μL diaminophthalhydrazide solution, heat at 80° for 110 min, cool, inject a 20 μL aliquot. (Prepare copper acetate solution by dissolving 700 mg copper(II) acetate in 10 mL water, dilute to 100 mL with MeOH, discard after 1 month. Diaminophthalhydrazide solution was 7.5 mM 4,5-diaminophthalhydrazide in 3.5 M hydrochloric acid containing 625 mM β-mercaptoethanol, discard after 5 h. Prepare 4,5-diaminophthalhydrazide dihydrochloride as follows. Reflux 316 g 4-nitrophthalic acid and 50 mL concentrated sulfuric acid in 500 mL MeOH for 10 h, recrystallize the product (dimethyl 4-nitrophthalate) from MeOH (mp 64-65E). Hydrogenate 47.8 g dimethyl 4-nitrophthalate in 300 mL MeOH over 13 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi. When the calculated amount of hydrogen has been absorbed remove the catalyst and evaporate to dryness under reduced pressure, recrystallize the residue from aqueous MeOH to give dimethyl 4-aminophthalate (mp 83-84E). Stir 146.3 g dimethyl 4-aminophthalate in 1.4 L acetic anhydride at 60-70E for 2 h then leave over-

night, precipitate product with MeOH. Dry the product and rinse it with sodium carbonate solution, re-dry, recrystallize from benzene/MeOH (Caution! Benzene is a carcinogen!) to give dimethyl 4-acetamidophthalate (mp 138-140E). Add 100.4 g to 600 mL fuming (90%) nitric acid at 0-5E over 30 min, stir at 5-10E for 2.5 h, mix the reaction mixture with 800 mL cold dichloromethane, shake with crushed ice. Remove the organic layer and extract the aqueous layer with 200 mL cold dichloromethane. Combine the organic layers and wash them with ice water, cold sodium bicarbonate solution, and cold water. Dry over anhydrous magnesium sulfate, evapor ate to dryness under reduced pressure and, recrystallize repeatedly from MeOH to give dimethyl 4-acetamido-5-nitrophthalate (mp 123-124.5E). Hydrolyze dimethyl 4-acetamido-5-nitrophthalate to dimethyl 4-amino-5-nitrophthalate. Hydrogenate 20.3 g dimethyl 4-amino-5-nitrophthalate in 250 mL MeOH over 1 g 5% platinum on carbon at an initial hydrogen pressure of 50 psi, remove the catalyst, evaporate to dryness under reduced pressure at 25E, recrystallize from chloroform/dichloromethane to give dimethyl 4,5-diaminophthalate (mp 111.5-113E). Add 1.1 g dimethyl 4,5-diaminophthalate to 3 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) and 3 mL triethylamine in 20 mL MeOH, concentrate the resulting solution, triturate with benzene/MeOH, recrystallize from N,N'-dimethylacetamide/acetic acid to give 4,5-diaminophthalhydrazide (6,7-diamino-2,3-dihydrophthalazine-1,4-dione) (mp 407E) (J. Heterocycl. Chem. 1973, 10, 891), mix 4,5-diaminophthalhydrazide with a small amount of concentrated HCl, recrystallize from EtOH to give 4,5-diaminophthalhydrazide dihydrochloride.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSKgel ODS-120T (Tosoh, Japan)
Mobile phase: MeCN:tetrahydrofuran:100 mM pH 7.0 phosphate buffer 24:3:73
Flow rate: 1.0
Injection volume: 20
Detector: Chemiluminiscence following post-column reaction. The column effluent mixed with 20 mM hydrogen peroxide in water pumped at 1.0 mL/min and then with 30 mM potassium hexacyanoferrate(III) in 3.0 M NaOH pumped at 2.0 mL/min and flowed to the detector.

CHROMATOGRAM

Retention time: 52
Internal standard: beclomethasone

OTHER SUBSTANCES

Extracted: dexamethasone
Simultaneous: aldosterone, corticosterone, cortisone, 11-deoxycortisol, hydrocortisone, 18-hydroxycorticosterone, 18-hydroxydeoxycorticosterone, prednisolone, prednisone
Noninterfering: androstendione, cholesterol, estrone, estradiol, estriol, pregnenolone, progesterone
Interfering: betamethasone.

KEY WORDS

plasma; derivatization; beclomethasone is IS

REFERENCE

Ishida,J.; Sonezaki,S.; Yamaguchi,M.; Yoshitake,T., Determination of dexamethasone in plasma by high-performance liquid chromatography with chemiluminiscence detection, *Anal.Sci.*, **1993**, *9*, 319–322.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 750 μL 40 μg/mL IS in EtOH, add 8 mL dichloromethane, extract on a roller mixer for 30 min. Centrifuge at 2500 rpm for 10 min at 25°, collect the organic layer, evaporate to dryness at 30° under a stream of nitrogen. Reconstitute the residue in 1 mL mobile phase, centrifuge at 15000 rpm for 2 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Alltima C18 (Alltech)
Mobile phase: MeCN:MeOH:water:glacial acetic acid 8.8:65:26.2:0.175
Flow rate: 1.3
Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 15.4 (dipropionate)
Internal standard: dexamethasone-21-acetate (5.2)

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
plasma; serum

REFERENCE
Foe,K.; Brown,K.F.; Seale,J.P. Decomposition of beclomethasone propionate esters in human plasma, *Biopharm.Drug Dispos.*, **1998**, *19*, 1–8.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL 20 μg/mL cloprednol + 3 mL ether, shake 10 min, centrifuge at 3000 g, remove the organic phase and evaporate it to dryness under nitrogen. Take up the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: Nucleosil R 10 C 18
Mobile phase: MeOH:MeCN:water:acetic acid 400:100:200:1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: cloprednol
Limit of detection: 500 ng/mL

KEY WORDS
for beclomethasone dipropionate; plasma

REFERENCE
Würthwein,G.; Rohdewald,P. Activation of beclomethasone dipropionate by hydrolysis to beclomethasone-17-monopropionate, *Biopharm.Drug Dispos.*, **1990**, *11*, 381–394.

SAMPLE
Matrix: blood, tissue
Sample preparation: Acidify plasma or lung tissue homogenate to pH 2 with 500 mM HCl, add 100 μL 20 μg/mL IS, extract with 8 mL dichloromethane. Evaporate the organic layer to dryness under vacuum, reconstitute in 120 μL MeOH:5% acetic acid 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS C18
Mobile phase: MeCN:MeOH:water 44:11:45
Flow rate: 1
Injection volume: 80
Detector: UV 242 or radioactivity

CHROMATOGRAM
Internal standard: hydrocortisone 21-S-propionate (JO 498)

OTHER SUBSTANCES
Extracted: metabolites, budesonide

KEY WORDS
for beclomethasone dipropionate; plasma; rat; lung; radiolabeled; pharmacokinetics

REFERENCE
Chanoine,F.; Grenot,C.; Heidmann,P.; Junien,J.L. Pharmacokinetics of butixocort 21-propionate, budesonide, and beclomethasone dipropionate in the rat after intratracheal, intravenous, and oral treatments, *Drug Metab.Dispos.*, **1991**, *19*, 546–553.

SAMPLE
Matrix: ileostomy effluent
Sample preparation: Dilute ileostomy effluent 1:2 by weight with water and mix with 100 µL 11 µg/mL 17-hydroxyprogesterone. Extract 3 g aliquot three times with 10 mL dichloromethane by shaking for 1 min and centrifuging at 2000 rpm for 2 min. Wash combined extracts successively with 2 mL 0.1 M NaOH and 4 mL water by shaking for 30 s and centrifuging for 1 min then dry the organic layer under air at 40°. Take up the extract in 1 mL MeOH, add 1.1 mL water and apply to C18 Bond Elut SPE cartridge. Wash with 10 mL water, wash with 5 mL MeOH:water 45:55, elute with 2 mL MeOH. Add 50 µL 20 µg/mL progesterone to the eluate, dry at 40°, take up in 100 µL MeOH, inject 10 µL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:50 mM pH 3.0 sodium phosphate buffer 55:45
Flow rate: 3
Injection volume: 10
Detector: UV 254 and 238

CHROMATOGRAM
Retention time: 21.3 (beclomethasone dipropionate)
Internal standard: 17-Hydroxyprogesterone (6.0) and progesterone (11.6)

OTHER SUBSTANCES
Extracted: beclomethasone alcohol, beclomethasone 17-monopropionate

KEY WORDS
SPE

REFERENCE
Levine,D.S.; Raisys,V.A.; Ainardi,V. Coating of oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active antiinflammatory drug to the terminal ileum, *Gastroenterology*, **1987**, *92*, 1037–1044.

SAMPLE
Matrix: tissue
Sample preparation: 100 mg Tissue + 2 mL Ringer's pH 6.8 phosphate buffer + 2 mL EtOH, centrifuge, wash residue twice. Pool supernatant and washings and evaporate to dryness, take up in 400 µL EtOH, inject an aliquot.

HPLC VARIABLES
Guard column: Used but not specified

Column: μBondapak C18
Mobile phase: Gradient. MeOH:water 40:60 to 80:20, time not specified
Flow rate: 1.5
Detector: UV 254

OTHER SUBSTANCES

Extracted: beclomethasone monopropionate, beclomethasone, cyclomethasone

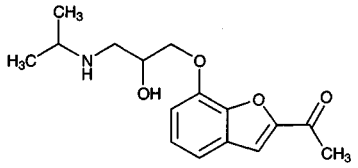
KEY WORDS

for beclomethasone dipropionate; lung

REFERENCE

Ronca-Testoni,S. Hydrolysis of cyclomethasone by the human lung, *Int.J.Clin.Pharmacol.Res.*, **1983**, *3*, 17–20.

Befunolol



Molecular formula: $C_{16}H_{21}NO_4$
Molecular weight: 291.35
CAS Registry No.: 39552-01-7
Merck Index: 1050

SAMPLE

Matrix: perfusate
Sample preparation: 50 µL Perfusate + 50 µL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 µL 50 µg/mL salicyl methionine in MeOH, centrifuge at 12000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 500

CHROMATOGRAM

Internal standard: salicyl methionine

KEY WORDS

rabbit

REFERENCE

Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

SAMPLE

Matrix: solutions
Sample preparation: 50 µL Solution + 50 µL pH 7.4 PBS + 100 µL 50 µg/mL salicylmethionine in MeOH, centrifuge at 12000 g for 10 min, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeOH:50 mM NaH_2PO_4 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 300 em 500

CHROMATOGRAM

Internal standard: salicylmethionine

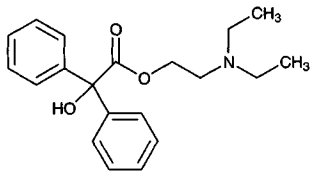
KEY WORDS

buffer; earle's balanced salt solution

REFERENCE

Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

Benactyzine



Molecular formula: $C_{20}H_{25}NO_3$
Molecular weight: 327.42
CAS Registry No.: 302-40-9, 57-37-4 (HCl)
Merck Index: 1055
Lednicer No.: 1 93

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 1.99

OTHER SUBSTANCES

Simultaneous: buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

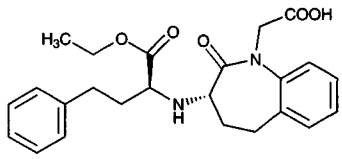
KEY WORDS

SFC; pressure 200 bar

REFERENCE

Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

Benazepril



Molecular formula: $C_{24}H_{28}N_2O_5$
Molecular weight: 424.50
CAS Registry No.: 86541-75-5, 86541-74-4 (HCl)
Merck Index: 1058
Lednicer No.: 5 135

SAMPLE

Matrix: formulations
Sample preparation: Add MeOH:water 50:50 to powdered capsules or tablets so as to give a benazepril concentration of ca. 20 μg/mL, stir for 15 min, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:THF:20 mM pH 2.5 sodium heptanesulfonate 45.6:2.4:52
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM

Retention time: 15.5

OTHER SUBSTANCES

Simultaneous: ramipril

KEY WORDS

capsules; tablets

REFERENCE

Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 17.003

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder. Weigh out an amount equivalent to 25 mg benazepril, extract with MeOH, filter. Mix 100-500 μL filtrate with 200 μL 4 mg/mL IS in MeOH, make up to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 211

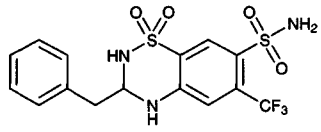
CHROMATOGRAM
Retention time: 18.53
Internal standard: enalapril (8.58)
Limit of detection: 5 μg/mL
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: cilazapril

KEY WORDS
tablets

REFERENCE
Gumieniczek,A.; Przyborowski,L. Determination of benazepril and cilazapril in pharmaceuticals by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2135–2142.

Bendroflumethiazide



Molecular formula: $C_{15}H_{14}F_3N_3O_4S_2$
Molecular weight: 421.42
CAS Registry No.: 73-48-3
Merck Index: 1064
Lednicer No.: 2 358

SAMPLE

Matrix: blood
Sample preparation: 3 mL Plasma + 2 mL 10 mM NaOH + 2 mL 10 mM HCl, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 50 µL 10 mM NaOH, vortex for 25 s, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: Isopropanol:water:acetic acid 17:82:1
Flow rate: 2
Injection volume: 20
Detector: UV 269

CHROMATOGRAM

Retention time: 14.4
Internal standard: bendroflumethiazide

OTHER SUBSTANCES

Extracted: trichlormethiazide

KEY WORDS

plasma; silanize glassware; bendroflumethiazide is IS

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 18.632

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in solvent, inject an aliquot. (Solvent was 750 mg KCl in 10 mL 1 M HCl, add 400 mL water, add 400 mL MeOH, make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 4 7 μm Nucleosil-100 phenyl
Column: 300 × 4 7 μm Nucleosil-100 phenyl
Mobile phase: MeOH:water 40:60
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: hydroflumethiazide, degradation products

REFERENCE
Frontini,R.; Mielck,J.B. Determination and quantitation of bendroflumethiazide and its degradation products using HPLC, *J.Liq.Chromatogr.*, **1992**, *15*, 2519–2528.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3001 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 1
Injection volume: 20
Detector: UV 272

CHROMATOGRAM
Retention time: 19, 21.5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 80:20, inject a 6 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 10 μm LiChrosorb RP-8
Column: 100 × 4.6 5 μm Spheri RP-18 (Brownlee)
Mobile phase: MeOH:water 80:20 containing 2 g/L lithium perchlorate
Flow rate: 0.5
Injection volume: 6
Detector: E, ESA Model 5100A Coulochem, model 5020 guard cell +950 mV, Model 5010 analytical cell + 400 mV, palladium reference electrode, following post-column photolysis. The effluent from the column flowed through a 20 m × 0.3 mm coil of PTFE tubing and was irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp.

CHROMATOGRAM
Retention time: 5
Limit of detection: 267 ng/mL

OTHER SUBSTANCES
Also analyzed: butizide, chlorthalidone, ethacrynic acid, furosemide, hydrochlorothiazide

KEY WORDS
post-column reaction

REFERENCE
Macher,M.; Wintersteiger,R. Improved electrochemical detection of diuretics in high-performance liquid chromatographic analysis by postcolumn on-line photolysis, *J.Chromatogr.A*, **1995**, *709*, 257–264.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica
Mobile phase: Heptane:isopropanol:diethylamine 80:20:0.1
Flow rate: 1
Injection volume: 1000
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.34

KEY WORDS
chiral; α 1.17

REFERENCE
Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 500 mg solid sodium bicarbonate, mix, add 2 mL 10 mM NaOH, mix, add 10 mL diethyl ether, shake gently on a platform shaker for 15 min, centrifuge at -10° at 2200 g for 15 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70° for 45 min (these conditions are required to remove residual benzyl alcohol that is present as a preservative in the heparin), reconstitute the residue in 100 μL MeOH, vortex for 25 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 5:35:59:1
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 10.7
Internal standard: bendroflumethiazide

OTHER SUBSTANCES

Extracted: trichlormethiazide

KEY WORDS

silanize glassware; bendroflumethiazide is IS

REFERENCE

Meyer,M.C.; Hwang,P.T.R. Determination of trichlormethiazide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *223*, 466–472.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM

Retention time: 6.8
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES

Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, clopamide, methyclothiazide, furosemide, metolazone, mefruside, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, in-

domethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE

Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 15 (A), 15.4 (B)

Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

Interfering: polythiazide

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 1 mL 10 mM HCl + 2000 ng bendroflumethiazide, extract with 5 mL ethyl acetate, centrifuge at 3000 rpm for 5 min. Remove the organic layer and dry it under a stream of nitrogen at 40°. Reconstitute with 100 μL MeOH, inject a 2 μL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: Gradient. MeOH: 50 mM ammonium acetate from 10:90 to 60:40 over 10 min, maintain at 60:40 for 10 min.
Column temperature: 40
Flow rate: 0.3
Injection volume: 2
Detector: F ex 223 em 415 or UV 230

CHROMATOGRAM
Retention time: 8.6
Internal standard: bendroflumethiazide

OTHER SUBSTANCES
Extracted: bumetanide (F ex 231 em 426 or UV), furosemide (UV), piretanide (UV), cyclopenthiazide (UV), etozolin (UV), canrenone (UV)

KEY WORDS
bendroflumethiazide is IS

REFERENCE
Gradeen,C.Y.; Billay,D.M.; Chan,S.C. Analysis of bumetanide in human urine by high-performance liquid chromatography with fluorescence detection and gas chromatography/mass spectrometry, *J.Anal.Toxicol.*, **1990**, *14*, 123–126.

SAMPLE
Matrix: urine
Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL MeCN/water, inject a 10-20 µL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 µm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 4.5:10.5:85:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Extracted: amiloride, chlorthalidone, triamterene, furosemide, benzthiazide

REFERENCE
Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE
Matrix: urine
Sample preparation: Direct injection.

HPLC VARIABLES
Guard column: 35 × 4 5 µm Spherisorb ODS-2
Column: 120 × 4 5 µm Spherisorb ODS-2
Mobile phase: MeOH:50 mM sodium dodecyl sulfate 5:95

Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 224

CHROMATOGRAM
Retention time: 15.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: chlorthalidone

REFERENCE
Bonet Domingo,E.; Medina Hernández,M.J.; Ramis Ramos,G.; Garcia Alvarez-Coque,M.C. High-performance liquid chromatographic determination of diuretics in urine by micellar liquid chromatography, *J.Chromatogr.*, **1992**, *582*, 189–194.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18
Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 270

CHROMATOGRAM
Retention time: 18.8
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, spironolactone, triamterene, trichlormethiazide

KEY WORDS
column-switching; optimum detection wavelengths vary for each drug

REFERENCE
Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Recon-

stitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: 7.0
Internal standard: 7-propyltheophylline (4.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: xipamide, bumetanide, acetazolamide, amiloride, benzthiazide, buthiazide, caffeine, canrenone, chlorthalidone, clopamide, cyclothiazide, diclofenamide, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, probenecid, spironolactone, torsemide, triamterene
Interfering: polythiazide, ethacrynic acid

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine
Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 20 ng/mL.

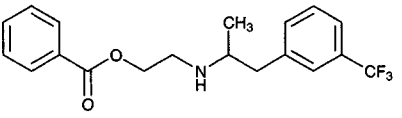
OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

Benfluorex



Molecular formula: $C_{19}H_{20}F_3NO_2$
Molecular weight: 351.37
CAS Registry No.: 23602-78-0, 23642-66-2 (HCl)
Merck Index: 1066

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.378

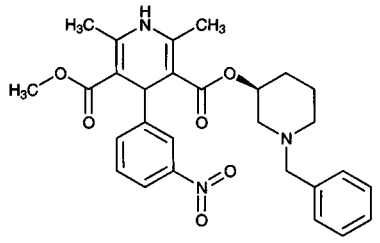
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Benidipine



Molecular formula: $C_{28}H_{31}N_3O_6$
Molecular weight: 505.57
CAS Registry No.: 105979-17-7
Merck Index: 1071

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Urine, bile. Inject urine and bile directly. Plasma. Add an equal volume of MeCN, centrifuge, remove supernatant and evaporate it to dryness. Reconstitute residue in MeCN:10 mM pH 5 ammonium acetate 20:80.

HPLC VARIABLES

Column: 300 × 8 Unisil Q5C18 (Gaschro Kogyo)
Mobile phase: Gradient. A was MeCN:10 mM pH 5 ammonium acetate 20:80. B was MeCN:10 mM pH 5 ammonium acetate 80:20. A:B from 100:0 to 92:8 over 4 min, to 83:17 over 4 min, to 75:25 over 4 min, to 63:37 over 4 min, to 55:45 over 8 min, to 50:50 over 8 min, to 42:58 over 4 min, to 0:100 over 9 min, hold at 0:100 for 25 min.
Flow rate: 2 for 36 min, then 3
Detector: Radioactivity

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; semi-preparative; rat; dog; radiolabelled

REFERENCE

Kobayashi,H.; Okumura,S.; Kosaka,Y.; Kobayashi,S.; Inoue,A.; Oka,T.; Nakamizo,N. Identification of benidipine hydrochloride metabolites in rats and dogs, *Arzneimittelforschung*, **1988**, *38*, 1753–1756.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 50 mg in 50 mL MeOH:water 70:30, remove a 10 mL aliquot and add it to 10 mL 1 mg/mL diphenylamine in MeOH, make this mixture up to 100 mL with MeOH:water 70:30, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:100 mM pH 5 ammonium acetate 70:30 (MeOH:50 mM pH 3.5 phosphate buffer:diisopropylamine 60:40:1 at 0.7 mL/min to detect other diastereomer)
Flow rate: 1
Injection volume: 10
Detector: UV 236

CHROMATOGRAM

Retention time: 10
Internal standard: diphenylamine (6)

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Suzuki,H.; Ono,E.; Ueno,H.; Takemoto,Y.; Nakamizo,N. Physico-chemical properties and stabilities of the highly potent calcium antagonist benidipine hydrochloride, *Arzneimittelforschung*, **1988**, *38*, 1671–1676.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out solid dispersion or solid dispersion granules equivalent to 20 mg benidipine hydrochloride, add 200 mL water, 100 mM hydrochloric solution, or McIlvaine buffer (pH 4.0, 6.0), filter (0.2 μm), dilute with MeOH, inject an aliquot.

HPLC VARIABLES

Column: C18 (YMC-Pack ODS-A)
Mobile phase: MeOH:THF:50 mM pH 3.0 phosphate buffer 27:8:65
Flow rate: 1
Detector: UV 237

CHROMATOGRAM

Internal standard: benzoin

KEY WORDS

solid dispersions; solid dispersion granules

REFERENCE

Suzuki,H.; Miyamoto,N.; Masada,T.; Hayakawa,E.; Ito,K. Solid dispersions of benidipine hydrochloride. I. Preparations using different solvent systems and dissolution properties, *Chem.Pharm.Bull.*, **1996**, *44*, 364–371.

SAMPLE

Matrix: solutions

Sample preparation: Direct injection of a MeOH solution containing 200-1000 ng.

HPLC VARIABLES

Column: 250 × 4.6 Sumchiral OA-4500 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:10:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 33 (+), 42(-) (α = 1.30)

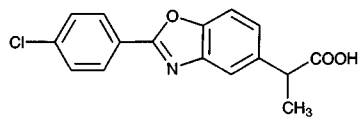
KEY WORDS

chiral

REFERENCE

Ohkubo,T.; Uno,T.; Sugawara,K. Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases, *J.Chromatogr.A*, **1994**, *659*, 467–471.

Benoxaprofen



Molecular formula: $C_{16}H_{12}ClNO_3$
Molecular weight: 301.73
CAS Registry No.: 67434-14-4
Merck Index: 1075

SAMPLE

Matrix: bile, blood
Sample preparation: 10 μL Plasma, or bile + 180 μL MeCN + 30 μL 100 μL/mL IS in DMSO + 30 μL water, vigorously mix. Centrifuge at 15000 g for 10 min at 4°, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 SUMICHIRAL OA 2500 (Sumika Chemical Analysis Service, Japan)
Column: 250 × 4.6 SUMICHIRAL OA 2500 (Sumika Chemical Analysis Service, Japan)
Mobile phase: MeOH containing 40 mM ammonium acetate (plasma) or MeCN:MeOH: water 15:85:5 containing 10 mM ammonium acetate (bile)
Flow rate: 1.0
Injection volume: 10
Detector: F ex 315, em 365

CHROMATOGRAM

Retention time: 10.3 (R, plasma) 11.8 (S, plasma), 22.5 (R, bile) 26.0 (S, bile)
Internal standard: naproxen methyl ester (4.4) (plasma), (4.3) (bile)
Limit of detection: 10 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; chiral; pharmacokinetics

REFERENCE

Mohri,K.; Okada,K.; Benet,L.Z. Stereoselective metabolism of benoxaprofen in rats. Biliary excretion of benoxaprofen taurine conjugate and glucuronide, *Drug Metab.Dispos.*, **1998**, *26*, 332–337.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: 10 μL Plasma, bile, or urine + 180 μL MeCN + 30 μL 100 μL/mL IS in DMSO + 30 μL water, mix vigorously. Centrifuge at 15000 g for 10 min at 4°, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Capcell Pak C18 (Shiseido, Japan)
Column: 250 × 4.6 5 μm Capcell Pak C18 (Shiseido, Japan)
Mobile phase: MeCN:THF:10 mM tetrabutylammonium hydrogen sulfate buffer 35:35:100
Flow rate: 1.3
Injection volume: 10
Detector: F ex 315 em 365

CHROMATOGRAM

Retention time: 17.5
Internal standard: naproxen methyl ester (13.2)

Limit of detection: 10 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Mohri,K.; Okada,K.; Benet,L.Z. Stereoselective metabolism of benoxaprofen in rats. Biliary excretion of benoxaprofen taurine conjugate and glucuronide, *Drug Metab.Dispos.*, **1998**, *26*, 332–337.